REMARKS

This response is being submitted within the shortened two-month statutory period set for responding to the October 9, 2001 document entitled "NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)." (A copy of the document entitled "NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)" is enclosed herewith for the Examiner's convenience.) Therefore, a fee for an extension of time is not required.

In response to the October 9, 2001 document entitled "NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)," we are enclosing herewith a document entitled "Sequence Listing," which is a formal sequence listing. A copy of the "Sequence Listing" is included on the enclosed computer-readable diskette. Also enclosed herewith is a document entitled "Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§ 1.821-1.825," which indicates that the formal written "Sequence Listing" does not include new matter and that the information recorded on the computer-readable diskette is identical to the formal written "Sequence Listing."

The enclosed formal written "Sequence Listing" numbers 18 sequences (SEQ ID NOS:1-18) that were not previously numbered in the originally filed U.S. patent application. Therefore, this response amends the third paragraph on page 28 of the specification to identify SEQ ID NOS:1-4; this response amends the second paragraph on page 30 of the specification to identify SEQ ID NO:5 and SEQ ID NO:6; this response amends the first paragraph on page 31 of the specification to identify SEQ ID NOS:7-10; this response amends the second paragraph on page 32 of the specification to identify SEQ ID NOS: 11-15; and this response

amends the first paragraph on page 33 of the specification to identify SEQ ID NOS: 16-18.

The Commissioner is authorized to charge any additional fees which may be required or credit overpayment to Deposit Account No. 12-0415. In particular, if this response is not timely filed, then the Commissioner is authorized to treat this response as including a petition to extend the time period pursuant to 37 C.F.R § 1.136(a) requesting an extension of time of the number of months necessary to make this response timely filed; and the petition fee due in connection therewith may be charged to deposit account No. 12-0415.

Respectfully submitted,

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Enclosures: A copy of the October 9, 2001 document entitled "NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)"

A document entitled "Sequence Listing"

Computer-readable diskette

A document entitled "Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§ 1.821-1.825"

Appendix A

APPENDIX A

RE: U.S. Patent Application No. 09/831,182
Applicant: Stefano COLLOCA
Title: "CELLS FOR THE PRODUCTION OF HELPER DEPENDENT ADENOVIRAL VECTORS, METHOD FOR THE PREPARATION AND USE THEREOF"
Our Ref.: B-4175PCT 618769-8

Please amend the third paragraph on page 28 of the specification (see lines 13-28 on page 28) as indicated below.

The E4 ORF6 gene DNA was amplified by PCR using the oligonucleotides 5'-TTATACGCGTGCCACCATGACTACGTCCG-3' (SEQ ID NO: 1) and 5'-TTATGCTAGCGCGAAGGAGAAGTCCACG-3' (SEQ ID NO: 2) and pFG140 containing Ad5 viral genomic DNA as substrate. Amplified DNA was inserted into the pBI plasmid (Clontech) (Baron, U. et al. (1995) Nucleic Acids Res, 23 (17) 3605-3606) containing a bidirectional promoter consisting of a single tetracycline-responsive element (TRE) flanked by two cytomegalovirus (CMV) minimal promoters, between the restriction sites MluI and NheI, generating the pBI.E4 plasmid. pBI.E4 was modified by inserting between the restriction sites NotI and SalI, the DNA coding for the adenovirus E1 region obtained by PCR, using the oligonucletides 5'-ATGCGCGGCCGCTGAGTTCCTCAAGAGG-3' (SEQ ID NO: 3) and 5'-ATGCGTCGACCAGTACCTCAATCTGTATCTTC-3' (SEQ ID NO: 4), finally obtaining the pBI.E1/E4 construct (Fig.1).

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Please amend the second paragraph on page 30 of the specification (see lines 16-37 on page 30) as indicated below.

A DNA fragment containing the whole Adenovirus 5 E4 region was obtained cleaving the plasmid pBHG10 (Bett et al. Proc. Natl Acad Sci. 91:8802-8806; 1994) with SpeI and ClaI restriction enzymes. The isolated fragment was ligated in the pBluescript vector (Stratagene), between the restriction sites SpeI and ClaI yielding the plasmid pBSE4. Then pBSE4 was modified by inserting an eptamer of DNA binding sites for the Tet repressor into the unique Pac I restriction site, generating pBSE4-ept. The Tet eptamer DNA was amplified by PCR using the oligonucleotides 5'-CTGATTAATTAAATAGGCGTATCACGAGGCC-3' (SEQ ID NO: 5) and 5'-CTGACGATCGCGTACACGCCTACTC-3' (SEQ ID NO: 6) and the plasmid pUHD10.3 as DNA template. The Tet binding site was cloned into PacI restriction site of pBSE4, just upstream the E2 promoter. The final goal was the reduction of background expression of E2 promoter exploiting the silencing effect of tetracycline-controlled transcriptional silencer as described by Rittner (Rittner K., et al (1997) J. Virol. 71:3307-3311). Ad5 E4 region present in PBSE4-ept was then eliminated by digestion with MfeI and ClaI restriction enzymes, the vector DNA was gel-purified and ligated to a Tk-Hygromicyn B resistance expression

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Please amend the first paragraph on page 31 of the specification (see lines 1-10 on page 31) as indicated below.

cassette DNA obtained by PCR with the oligonucleotides 5'-AGTGCACAATTGATTTAAATAATCCGCGCGGTGG-3' (SEQ ID NO: 7) and 5'-TGCAATCGATCAACGCGGGCATCC-3' (SEQ ID NO: 8) using pCEP-4 plasmid DNA as template, generating pBSΔE4. The adenovirus ITRs in head-to-tail configuration were than amplified by PCR using the oligonucleotides 5'-TCGAATCGATACGCGAACCTACGC-3' (SEQ ID NO: 9) and 5'-TCGACGTGTCGACTTCGAAGCGCACACCAAAAACGTC-3' (SEQ ID NO: 10) and pFG140 (Microbix) plasmid DNA as template. The Ad ITRs were cloned into the NruI unique site of pBSΔE4, generating pBSΔE4J.

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Please amend the second paragraph on page 32 of the specification (see lines 7-37 on page 32) as indicated below.

Ad/EBV shuttle plasmids (pSA-1 and pSA-2) were further modified by deleting a DNA sequence corresponding to DNA binding protein (DBP) gene (nucleotides 22443-24032 of Ad5 sequence). The deletion was obtained by homologous recombination in E. coli, following the method described by A.F. Stuart and coworkers (Zhang et al. Nat. Genet. 1998;20:123-128). A DNA fragment containing the Tn5 kanamycin resistance gene (neo) flanked by DNA sequences was obtained by PCR with oligonucleotides 5'-GCGGTTAGGCTGTCCTTCTTCTCGACTGACTCCATGATCTTTTTCTGCCTATAGG AGAAGGAATCCCGGC GGATTTGTCCTACTCAGGAGAGCG-3' (SEQ ID NO: 11) and 5'-AAATGCTTTTATTTGTACACTCTCGGGTGATTATTTACCCCCACCCTTGCCGTCT GCGCCGTTCTGCAAACCCTATGCTACTCCGTCG-3' (SEQ ID NO: 12) consisting of about 60-bp of homology to DBP gene and, at 3' ends PCR primers to amplify neo gene using pGKfrt as template. Linear DNA containing neo gene was used in recombination experiments to delete the DBP gene from Ad/EBV shuttle plasmids. The same method was applied to construct Ad/EBV shuttle plasmids that do not express Ad polymerase gene and preterminal protein. The sequence of the oligonucleotides used to delete the polymerase gene was 5'-ACGGCCTGGTAGGCGCAGCATCCCTTTTCTACGGGTAGCGCGTATGCCTGCGC GGCCTTCCGGTCTGCAAACCCTATGCTACTCCGTCG-3' (SEQ ID NO: 13) and 5'-AGACCTATACTTGGATGGGGGCCTTTGGGAAGCAGCTCGTGCCCTTCATGCTG GTCATGTCCCGGCGGATTTGTCCTACTCAGGAGAGCG-3' (SEQ ID NO: 14). Two pairs of oligonucleotides were used to to delete two regions within the the main exon of pTP:5'-CCGCCTCCCGGTGCGCCGTCGTCGCCGCCGTGTCCCCCCTCCCCCACCGTC CCGGCGGATTTGTCCTACTCAGGAGAGCG-3' (SEQ ID NO: 15) and 5'-GATCTCCGC GTCCGGCTCGCTCCACGGTGGCGGCGAGGTCGTTGGAAATGCGTCTGC

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Please amend the first paragraph on page 33 of the specification (see lines 1-9 on page 33) as indicated below.

AAACCCTATGCTACTCCGTCG-3' (SEQ ID NO: 16),
5'-TCGACAGAAGCACCATGTCCTTGGGTCCGGCCTGCTGAATGCGCAGGCGGTCT
GCAAACCCTATGCTACTCCGTCG-3' (SEQ ID NO: 17), and
5'- TCGCCCCCGGAGCCCCGGCCACCCTACGCTGGCCCCTCTACCGCCAGCCGCTC
CCGGCGGATTTGTCCTACTCAGGAGAGCG-3' (SEQ ID NO: 18).
The same method can be applied to other region of Adenovirus genomic DNA relevant to obtain a reduction of cytotoxic effects produced by viral gene expression in the infected cell.